REMARKS

Reconsideration of this Application is respectfully requested.

Status of the Claims

Claims 65, 66, 68-71, 73 and 75-96 are pending in the application, with claim 65 being the sole independent claim.

Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

I. Claim Rejections Under 35 U.S.C. § 103

A. Munro, Komoriya and Tang

Claims 65-66, 68-71, 73, 75 and 81-96 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Munro, *Trends in Cell Biol. 8*:11-15 (1998) ("Munro"), WO98/37226 ("Komoriya"), and Tang *et al., J. Biol. Chem. 267*:10122-10126 (1992) ("Tang"). *See* Office Action, page 3. Applicants respectfully traverse this rejection.

The present claims are directed to compositions comprising a chimeric nucleic acid that encodes a polypeptide. The polypeptide comprises a first domain, a second domain and a third domain. The first domain comprises a Golgi apparatus retention signal or endoplasmic reticulum (ER) retention signal. The second domain comprises a protease cleavage site. The third domain comprises a reporter molecule.

The compositions encompassed by the present claims are useful for, *inter alia*, non-invasively monitoring the activity of Golgi- and/or ER-retained proteases in cells. *See* specification, pages 41-44 (Example 1). For example, the chimeric nucleic acid can be used to encode within a cell a polypeptide comprising: (1) a Golgi apparatus retention signal or an ER-retention signal; (2) a protease cleavage site; and (3) a reporter molecule. The polypeptide is confined to the Golgi or ER of the cell. Proteases that are localized to the Golgi or ER act on the protease cleavage site, thereby releasing the reporter molecule which can be detected, *e.g.*, in the extracellular medium. *See* specification, page 10, paragraph 34.

Cells that express the chimeric nucleic acid molecule of the invention can be used to non-invasively screen putative inhibitors of the Golgi- or ER-resident proteases. A compound that is shown to reduce or inhibit the release of the reporter molecule is identified, not only as a protease inhibitor, but also as a compound that can permeate the cell and enter the Golgi or ER compartment. *See* generally, specification, pages 3-4, paragraph 7. The ability of compounds to permeate the cell, enter the appropriate sub-cellular compartment and act on the resident protease is important if the compound is to be administered to subjects *in vivo*. *See id.*

Thus, the compositions of the present invention solve the previously intractable problem of *non-invasively* assaying the activity of Golgi- and ER-resident proteases and allowing the identification of effective inhibitors thereof. The solution to this problem derives from the novel and non-obvious combination of a Golgi or ER retention signal with a protease cleavage site and a reporter molecule.

It should be noted at the outset that none of the cited references acknowledges or makes reference to the need or desire of persons in the art to *non-invasively* assay proteases that are confined to the Golgi or ER. Likewise, none of the references allude to the need or desire of persons in the art to identify compounds that can permeate the cell, enter the Golgi or ER compartment, and act on Golgi- or ER-resident proteases. Therefore, a person of ordinary skill in the art, in view of the cited references, would have seen no need to target and confine a detectable protease substrate to the Golgi or ER. By logical extension, a person of ordinary skill in the art would have had no motivation to combine a Golgi or ER retention signal with a protease cleavage site and a reporter molecule.

To support the rejection, the Examiner has asserted that "[o]ne of ordinary skill in the art would have been motivated to [combine the cited references] in order to *detect, identify and or purify specific Golgi proteases* that cleaves the polypeptide during processing in Golgi." *See* Office Action, page 5 (emphasis added). Applicants first note that none of the cited references actually indicate or suggest a need in the art for detecting, identifying and/or purifying specific Golgi proteases. In addition, even if, *arguendo*, there were a recognized need in the art for detecting, identifying and/or

purifying specific Golgi proteases, a person of ordinary skill would nonetheless have had no motivation to combine a Golgi retention signal with a protease cleavage site and a reporter molecule. Routine purification techniques and assays, *i.e.*, techniques and assays *that do not involve the use of a polypeptide comprising a Golgi retention signal, a protease cleavage site and a reporter molecule*, were available in the art for detecting, identifying and/or purifying specific Golgi proteases. *See, e.g.*, Vey *et al.*, *J. Cell Biol. 127*:1829-1842 (1994) (copy submitted herewith as Exhibit 1, illustrating the isolation and characterization of the endogenous furin protease from MDBK cells using Golgi fractionation and chromatography methods). Thus, even if a skilled person were somehow interested in detecting, identifying and/or purifying specific Golgi proteases, as asserted by the Examiner, there would have been no reason whatsoever to combine a Golgi retention signal with a protease cleavage site and a reporter molecule.

As noted above, the compositions of the present invention are particularly suited for solving the specific problem of *non-invasively* assaying Golgi- and ER-resident proteases and identifying inhibitors thereof that are capable of permeating the cell and entering the Golgi and/or ER sub-cellular compartments. The cited references do not address or even contemplate this problem and therefore do not provide any motivation to combine a Golgi retention signal with a protease cleavage site and a reporter molecule.

Munro provides a broad overview of how proteins, in general, are localized to the Golgi apparatus. There is nothing in Munro, however, to suggest the need or desirability for reagents that are localized to the Golgi and that allow for the non-invasive detection of protease activity. There is certainly no suggestion in Munro to combine a Golgi retention signal with a protease cleavage site and a reporter molecule.

Komoriya refers to fluorogenic protease indicators that contain a protease binding site and fluorophores. *See* Komoriya, page 3, line 25 (Formula I). Komoriya mentions that the fluorogenic protease indicators can be used to detect protease activity in biological samples. *See* Komoriya, page 42, lines 9-10. Nothing in Komoriya, however, suggests that the indicators can be used to detect Golgi- (or ER-) resident proteases. In fact, all of the biological detection applications mentioned in Komoriya involve simply contacting a sample (*e.g.*, a tissue section) with an *exogenously added* fluorogenic

protease indicator. *See* page 42, lines 20-23, page 43, lines 14-17, and page 44, lines 19-21. In view of these proposed *in vitro*, *ex vivo*, and *in situ* applications, it would have made no sense to include a Golgi apparatus retention signal in the fluorogenic protease indicators of Komoriya. An exogenous protein containing a Golgi retention signal that is simply contacted with a cell *in vitro*, *ex vivo*, or *in situ* (as mentioned in Komoriya) would not localize to the Golgi apparatus. In the proposed biological applications in Komoriya, the exogenously added protease indicator -- even if it contained a Golgi retention signal -- would not be able to pass through the cell membrane and find its way to the intracellular sorting machinery that is responsible for targeting and localizing proteins to the Golgi. (Nothing in Komoriya suggests expressing a fluorogenic protease indicator in a cell from a chimeric nucleic acid molecule, a process which would at least allow for the possibility of delivering the indicator to sub-cellular compartments within the cell.)

Thus, not only is Komoriya silent as to the use of fluorogenic protease indicators to detect Golgi-resident proteases, but even if for some reason a Golgi apparatus retention signal were added to the protease indicators of Komoriya, the resultant indicators would not be functional for detecting Golgi-resident proteases when added exogenously to cells. Thus, a person of ordinary skill in the art would have had no motivation to modify the fluorogenic protease indicators of Komoriya.

Finally, Tang relates to the identification of the portions of the N-acetylglucosaminyltransferase I ("NT") protein that are responsible for its Golgi retention. *See* Tang, page 10122, right column. Tang refers to DNA constructs that express various chimeras containing N-terminal portions of NT fused to a reporter sequence (either dipeptidyl peptidase IV ("D4") or the transferin receptor ("TFR")). *See id.* The constructs of Tang were transfected into cells, and the transfected cells were analyzed by indirect immunofluorescence (using primary antibodies to D4 or TFR) in order to determine if the chimeric constructs localized to the Golgi. *See* Tang, pages 101122-101123. Thus, the sub-cellular localization of the reporter sequences indicated whether or not the respective portion of NT actually localized to the Golgi. *See, e.g.*, Tang, page 10124, Fig. 2.

In order for the experiments of Tang to provide meaningful results, maintenance of the *physical linkage* between the putative Golgi retention signals of NT and the reporter sequence was *essential*. If the reporter was somehow separated from the NT fragment, the sub-cellular localization of the reporter would provide no information as to the localization of the NT fragment, *i.e.*, whether or not it localized to the Golgi.

Since the experiments of Tang require the maintenance of the physical linkage of the reporter sequence to the NT fragment, it would have been entirely counterproductive and irrational to include a protease cleavage site in the chimeras of Tang. If one were to include a protease cleavage site in the constructs of Tang, it would be impossible to know whether the localization of the reporter sequence within the cell correlated with the localization of the NT portion. The sub-cellular localization of the reporter would be completely random if the protease cleavage site were cleaved, thereby separating the reporter from the NT portion.

Thus, there is nothing in Tang that would have suggested the inclusion of a protease cleavage site in the chimeras set forth therein. In fact, the addition of a protease cleavage site to the chimeras of Tang would completely defeat the purpose of the experiments.

As the discussion above indicates, Tang and Komoriya relate to two entirely distinct problems: Tang relates to the problem of identifying the portion of NT that is responsible for its Golgi retention. Komoriya relates to the problem of assaying protease activity in samples -- *in vitro*, *ex vivo*, or *in situ*. Neither of these references nor the problems sought to be addressed therein would have motivated one of ordinary skill in the art to produce a composition that falls within the scope of the present claims. Adding a protease cleavage site to the chimeras of Tang would have defeated the purpose of the experiments. Adding a Golgi retention signal to the reagent of Komoriya would have been pointless. Accordingly, a person of ordinary skill in the art would have had no motivation to combine or modify these references and, in fact, would have been discouraged from doing so.

Applicants therefore respectfully request that this rejection be reconsidered and withdrawn.

B. Munro, Komoriya, Tang, Steiner and Tomita

Claims 76-80 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Munro, Komoriya and Tang, and further in view of Steiner *et al.*, *FEBS Lett. 463*:245-249 (1999) ("Steiner") and Tomita *et al.*, *J. Biol. Chem. 273*:6277-6284 (1998) ("Tomita"). *See* Office Action, page 6. Applicants respectfully traverse this rejection.

Munro, Komoriya and Tang are discussed above. As explained above, one of ordinary skill in the art would have had no motivation to modify or combine these references, and in fact would have been discouraged from doing so. Likewise, a person of ordinary skill in the art would have had no motivation to modify or combine Steiner and/or Tomita with each other or with any of the other cited references.

Tomita simply relates to the general mechanism of APP cleavage by α-, β-, and γ-secretases. *See, e.g.*, Tomita, page 6277, abstract. Tomita says nothing about assaying Golgi-resident proteases, and certainly does not suggest a chimeric nucleic such as that which is encompassed by the present claims.

Steiner refers to a system for assaying non-native, exogenously expressed proteases in yeast cells. The Steiner system involves the use of a substrate that links the GAL4 transcription factor to a membrane via a caspase-3 recognition sequence. *See* Steiner, page 246, Fig. 1(a). Cleavage of the substrate by exogenously expressed human caspase-3 releases GAL4 from the membrane, thereby allowing it to enter the nucleus, interact with the GAL4 binding domain of a reporter construct and thereby activate expression of *lacZ*. *See id.* According to Steiner, yeast cells were specifically chosen for this system because:

many proteases typical for the specialized tasks of higher eukaryotic cells, like for example caspases, are absent in this organism. In addition, yeast does not contain homologues of Notch or AD associated genes, like βAPP or the PS.

See Steiner at page 245, middle right column (internal citations omitted).

Thus, the system of Steiner is specifically designed for assaying the activity of proteases that are *not normally expressed in yeast cells*. Including a Golgi apparatus retention signal in the GAL4 construct of Steiner would serve no evident purpose. If

anything, confining the constructs of Steiner to a sub-cellular compartment, such as the Golgi, would most likely be counterproductive since such confinement might prevent the GAL4 constructs from coming into contact with the expressed protease, thereby defeating the entire purpose of the assay system.

In explaining the rejection, the Examiner stated that:

[I]t would have been obvious to those skilled in the art to replace the GAL4 sequence (a yeast transcription initiator) in the chimeric polypeptide taught by Steiner et al. with a Golgi retention signal such as that taught by Tang et al. . . . One of ordinary skill in the art would have been motivated to replace the GAL4 sequence in the chimeric polypeptide of Steiner et al. as Tomita et al. teach that the APP is cleaved by α-, β-, and gamma-secretases in steps(s) during the transport of APP through Golgi complex.

See Office Action, page 7. As explained below, this statement cannot support a *prima facie* case of obviousness.

First, Applicants respectfully note that the Examiner's statement is logically flawed: It does not follow that one of ordinary skill in the art would have been motivated to replace GAL4 with a Golgi retention signal in the construct of Steiner simply because Tomita states that APP is cleaved by α-, β-, and γ-secretases during transport of APP through Golgi complex. The Examiner has not explained how the data from Tomita relate in any way to the asserted modification of the constructs of Steiner.

Second, replacing GAL4 with a Golgi retention signal in the construct of Steiner would render the system of Steiner completely inoperative. Without the GAL4 component, there would be no way to detect cleavage of the recognition signal by the protease. As noted above, the GAL4 component is essential to the system of Steiner. By interacting with the GAL4 binding domain on the *lacZ* reporter construct, GAL4 provides the only way that one can know whether or nor the protease cleavage site has been severed. Clearly, one of ordinary skill in the art would not have been motivated to replace the GAL4 component with a Golgi retention signal since this substitution would render the assay system of Steiner completely useless and would serve no apparent alternative purpose.

Finally, replacing GAL4 with a Golgi retention signal in the construct of Steiner would, in any event, result in a construct that is outside the scope of the present claims.

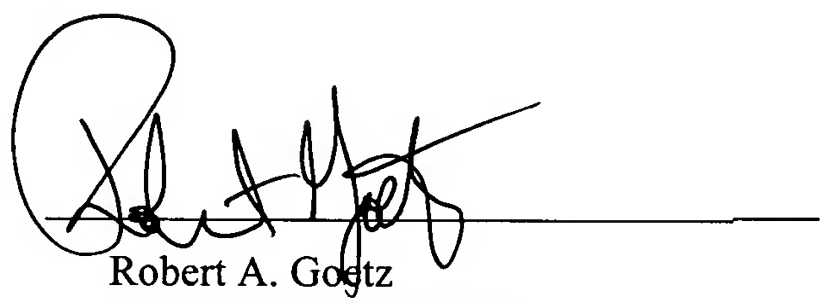
In summary, the Examiner has based this ground of rejection on the assertion that one of ordinary skill in the art would have been motivated to replace the GAL4 component of the chimeric polypeptide of Steiner with a Golgi retention signal because Tomita teaches that the APP is cleaved by α-, β-, and γ-secretases during transport of APP through Golgi complex. As noted above: (a) the substitution proposed by the Examiner does not logically follow from the results of Tomita; (b) the substitution proposed by the Examiner would render the system of Steiner totally useless; and (c) the substitution proposed by the Examiner would not result in subject matter that falls within the scope of the currently presented claims. Thus, Applicants respectfully request that this ground of rejection be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Dated: July 5, 2005



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